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RDA Workshop: New Approaches, Endpoints and Paradigms for RDAs of Mineral Elements

Functional Changes Appropriate for Determining Mineral Element Requirements[1,2]

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ABSTRACT One factor limiting efforts to determine human requirements for dietary intakes of mineral elements has been the unavailability of acceptable standards for evaluating the effects of marginal and mild deficiencies. Traditional approaches, such as growth, longevity, chemical balance and measurement of concentrations of minerals in plasma or serum and cellular components of the blood, have not been sensitive indicators of mineral nutriture. One alternative that has been shown to be responsive to graded dietary mineral intake is the evaluation of functional responses to specific challenges or stressors. Aberrant responses, either exaggerated or attenuated, to controlled stressors have been observed in a variety of physiological, psychological and immunological parameters when mineral intakes have been suboptimal by conventional standards, compared with adequate responses. In comparison to static biochemical approaches for assessment of mineral nutritional status, functional tests may be sensitive and responsive to alterations in mineral intake in adult humans. Dynamic functional measures complement static biochemical measures and reflect the net effect of deficiencies on integrated biological systems. The application of some of these types of dynamic evaluations of function may be a useful and productive approach for proposing mineral element intakes to optimize human health and biological function and performance. J. Nutr. 126: 2354S–2364S, 1996.

INDEXING KEY WORDS:

• *behavior* • *blood pressure* • *energy expenditure* • *humans* • *thermoregulation*

Despite increasing interest in defining the basic biological roles of the mineral elements and understanding how these elements function in the etiology of chronic diseases, the ability to define dietary intakes of minerals to optimize human health and various aspects of biological function is quite limited. This limitation cannot be ascribed to a lack of available analytical methods to measure mineral concentrations in various biological specimens for the assessment of mineral nutritional status because acceptable analytical resources are available. However, the basic clinical understanding of how to utilize these analytical capabilities for the measurement of the mineral nutritional status of an individual is lacking. This fundamental problem indicates the need to examine mineral nutritional status assessment from a different experimental perspective.

Traditionally, assessment of trace element nutritional status has included indices of growth and longevity but recently has relied on the measurement of mineral element concentrations in the fluid, components and cells in the blood. This strategy is based on the assumption that circulating mineral concentrations reflect organ and tissue mineral contents. Although this approach has been useful for identifying individuals

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with graded deficiencies of iron nutriture (Dallman 1986), it has not been a sensitive method for the evaluation of the nutritional status of other mineral elements. Other biochemical indicators of mineral element nutriture, such as the activities of mineral-dependent enzymes in the circulation, also have been insensitive to marginal and mild mineral depletion and unresponsive to short-term mineral supplementation. These limitations suggest that another experimental strategy may be required. One alternative is the use of functional tests for assessment of human nutritional status.

The concept of functional testing is quite broad and can involve any measurable aspect of biological function. Many systems have been suggested as areas of investigation including structural integrity, host defense, transport, hemostasis, reproduction, nerve function, work capacity and cognitive performance (Solomons and Allen 1983). Regardless of the test, impaired nutritional status is defined as the dietary intake which results in depression of physiological and psychological function and performance.

It is important to recognize that function per se is a global assessment. That is, any perturbation of response to a challenge affects the performance of integrated biological systems and subsystems. It is evident that the consequence of insufficient mineral element intake or body stores of the nutrient is the ultimate failure of one or more biological processes which initially is manifest as malfunction (exaggeration or attenuation of response) in a single system component but over time will result in multisystem impairment. The loss of such functions is biologically more important to the individual than the concentrations of a mineral element in the circulation or in a tissue or organ. Therefore, functional indicators of mineral element nutritional status may serve as diagnostic tests to determine the sufficiency of mineral element nutriture to permit cells, tissues, organs, anatomical systems, and the individual to perform optimally the nutrient-dependent biological function.

The concept of compensation is inherent in the use of functional assessment of mineral element nutriture. Briefly, when an individual is exposed to a controlled stressor (e.g., exercise, temperature, and mental task), the measured biological response, which is hypothesized to be dependent on a specific mineral, is regulated by the degree of sufficiency of the body or tissue store of that mineral element. If stores are adequate, the individual responds without an excessive or impaired reaction. However, when presented with the same stressor, the biological response is adverse or deleterious if mineral nutritional status is suboptimal.

Studies of animals fed diets containing varied amounts of mineral elements ranging from adequate to severely deficient provide insight into which biological functions might be evaluated as indicators of altered nutritional status. Mineral elements are involved in many biological functions. They directly influence endocrine, immune and central nervous system functions which affect metabolism and psychological function and performance. Through their influences on neuroendocrine and immune systems, the mineral elements also affect behavior. This conceptual framework provides many experimental opportunities to examine and evaluate biological function and performance in response to controlled studies of dietary manipulation of mineral elements.

The purpose of this review is to delineate alterations in human physiological, psychological and immune function and performance in response to controlled manipulations of dietary mineral elements. Comparisons of responses in biochemical assessments of mineral nutritional status with functional changes will be made.

PHYSIOLOGICAL FUNCTION

Knowledge of the biological roles supported by mineral elements provides some insight into the potential variables that should be monitored in controlled studies of human mineral element depletion and repletion. The use of well-defined and dynamic tests of integrated physiological functions permits a novel strategy for evaluating the impact of mineral nutrient intakes in humans. Studies of animals severely deprived of individual mineral elements provide insight into experimental approaches that might be used to identify physiological variables and dynamic functions that might be examined.

Resting metabolic rate and zinc

A basic physiological parameter that responds to nutritional deprivation is basal or resting energy expenditure. In a 75-d study, resting metabolic rate was determined in six men fed diets containing 16.4, 5.5 and 16.5 mg zinc daily for 12, 54 and 9 d, respectively (Wada and King 1986). Low dietary zinc was associated with a slight but significant decrease in resting metabolic rate (**Table 1**) that was paralleled by a significant decrease in free thyroxine without similar trends in total thyroxine (T4) and triiodothyronine (T3). Zinc status, as assessed with serum zinc concentrations, did not change significantly. Thus, impaired biological function was identified independently of changes in usual indices of zinc nutriture when graded amounts of dietary zinc were fed to men.

Copper and blood pressure response to isometric work

Studies of adult rats fed diets deficient in copper have found hypertension (Klevay 1987, Mederios 1987). Confirmation of altered blood pressure regulation in

TABLE 1

Resting metabolic rate (RMR) and thyroid hormones in men fed graded dietary zinc[1,2]

Dietary zinc, *mg/d*	16.4	5.5	5.5	16.5
Study day	11	28	66	63
RMR, *kJ/(kg·d)*	4.18 ± 0.08[a]	4.10 ± 0.29[a]	3.81 ± 0.20[b]	3.97 ± 0.20[ab]
T_4[3], *nmol/L*	109 ± 11	85 ± 7	90 ± 5	100 ± 11
FT_4, *nmol/L*	18.7 ± 0.7[ac]	14.9 ± 0.7[b]	15.8 ± 0.4[bc]	19.0 ± 1.4[a]
T_3, *nmol/L*	2.9 ± 0.2	3.0 ± 0.3	3.0 ± 0.2	3.3 ± 0.3

1 Adapted from Wada and King (1986).
2 RMR and thyroid hormone values are means ± SE. [abc] Values in same row with different superscripts are significantly different ($P < 0.05$).
3 T_4 = total thyroxine, FT_4 = free thyroxine, T_3 = triiodothyronine.

adult humans fed diets low in copper has been lacking until the addition of a controlled stressor was utilized.

A group of young women lived on a metabolic unit and were fed diets made of conventional foods and varying in copper content (Lukaski et al. 1988). An initial control diet adequate in copper (1.45 mg/d) was fed for 14 d, followed by a diet low in copper (0.65 mg/d) for 84 d that was supplemented with copper (2.65 mg/d) for 37 d.

Autonomic cardiovascular function was assessed noninvasively at the end of each dietary period by using standard tests of increasing stressor intensity. No effects of dietary copper on heart rate and blood pressure responses when low intensity stressors, resting in a supine position and moving from a supine to an upright position, were observed. However, an exaggerated blood pressure response to standardized hand-grip exercise (30% maximal voluntary contraction) was observed when dietary copper was restricted to 0.65 mg/d. Systolic was greater ($P < 0.05$) after 3 min and diastolic pressure was greater ($P < 0.05$) after 2 min of hand-grip exercise (**Fig. 1**). Mean arterial pressure, which is an integrated measure of blood pressure that includes contributions from both myocardial and arterial components of the cardiovascular system, was increased ($P < 0.05$) after 1 min of isometric work when dietary copper was 0.65 mg/d (**Fig. 2**).

Biochemical evidence of copper deficiency was limited. Copper balance, exclusive of surface losses, was −0.04 ± 0.03 (mean ± SE) mg/d when dietary copper was 0.65 mg/d. In contrast, copper balance was 0.02 ± 0.05 mg/d when copper intake was 2.65 mg/d. Although there was a qualitative difference in copper retention (e.g., negative with low copper and positive with supplemental copper), it was not statistically different because of the variability in the balance data both within and between individuals. Similarly, plasma copper concentrations were unaffected by dietary copper. However, ceruloplasmin diamine oxidase (EC 1.16.3.1) activity in plasma decreased ($P < 0.05$) with low dietary copper. The ratio of enzymatic to immunoreactive ceruloplasmin decreased ($P < 0.05$) more than 20% when dietary copper was restricted.

Because both biochemical indices of copper status and physiological function apparently were influenced by dietary copper, their relationship was examined. Individual volunteer ceruloplasmin ratios and the mean arterial pressures at the end of the sustained hand-grip tests were examined with linear regression analysis (**Fig. 3**). With the exception of one volunteer in whom this relationship was weak, the individual correlation coefficients ranged from −0.90 to −0.99. Overall, the ceruloplasmin ratio was a significant predictor of mean arterial pressure at the end of 5 min of isometric work

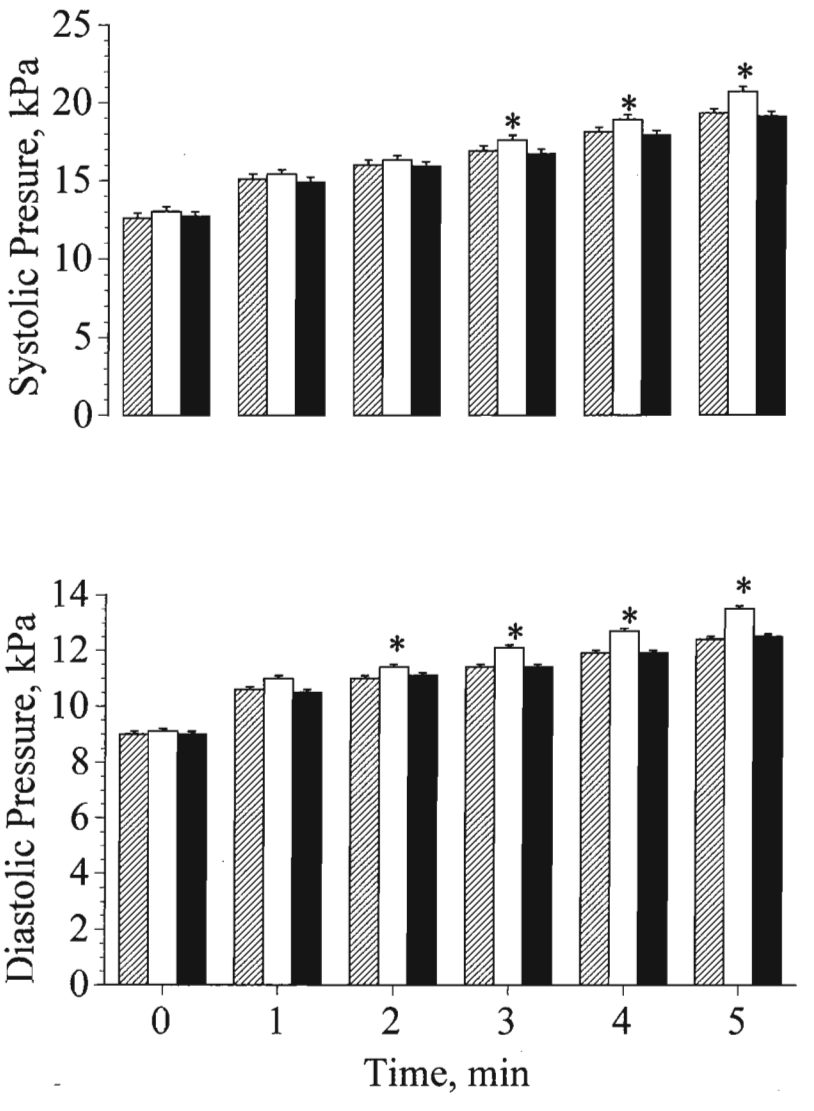

FIGURE 1 Effects of dietary copper (Cu) on systolic and diastolic blood pressure responses to isometric hand-grip exercise. Partially shaded bars = 1.45 mg Cu/d, open bars = 0.65 mg Cu/d and fully shaded bars = 2.65 mg Cu/d. Asterisks indicate significant differences ($P < 0.05$) by repeated measures ANOVA and Tukey post-hoc test. Used with permission from Lukaski et al. 1988.

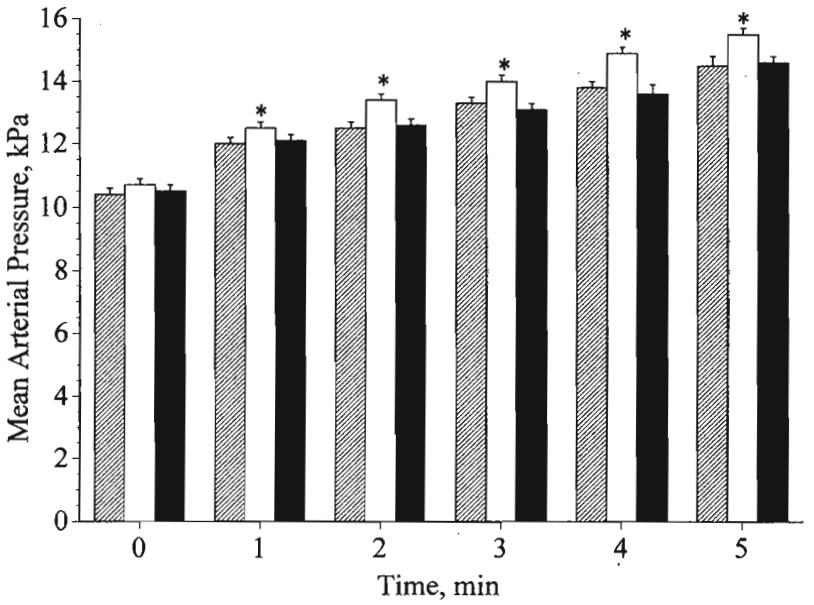

FIGURE 2 Effects of dietary copper (Cu) on mean arterial pressure at the end of 5 min of hand-grip work. Partially shaded bars = 1.45 mg Cu/d, open bars = 0.65 mg Cu/d and fully shaded bars = 2.65 mg Cu/d. Asterisks indicate significant differences ($P < 0.05$) by repeated measures ANOVA and Tukey post-hoc test. Used with permission from Lukaski et al. 1988.

during the hand-grip test. This finding demonstrates a relationship between a biochemical index of copper nutriture and an altered physiological response to a controlled stressor when copper nutriture was changed.

Iron, thermogenesis and energy expenditure

Detailed studies in rats clearly indicate a critical role of iron nutriture in the regulation of body temperature and energy production in response to reduced environmental temperature (Beard et al. 1984, Smith and Lukaski 1992). These findings have been extended in humans in some controlled studies.

One early report suggested a deleterious effect of reduced iron on physiologic function during acute cold exposure. Martinez-Torres et al. (1984) observed a graded reduction in oral temperature of men who were anemic, iron-deficient without anemia and iron-adequate during 1 h of immersion in 20°C water. Additionally, oxygen consumption was increased during the cold exposure in response to decreased iron status. The investigators hypothesized that the decreased body temperature was attributable to an increased heat loss during immersion in the cold water.

These initial findings were expanded by two subsequent human studies. Thermoregulatory function in cold air (16°C) was studied in young women who resided on a metabolic unit and experienced iron depletion by consumption of controlled dietary iron and phlebotomy and iron repletion by diet and iron supplementation (Lukaski et al. 1990). Iron depletion was associated with impaired physiological function, including significantly decreased rate of metabolic heat production (49.6 ± 1.1 vs. 53.6 ± 1.2 W/m^2), increased rates of cooling of internal body (−0.4 vs. −0.13°C/h) and skin (−3.2 vs. −2.1°C/h) temperatures, a shift in the lower core temperature threshold for shivering and an earlier onset on shivering (**Fig. 4**).

Circulating hormone concentrations also were affected by changes in iron status. The anticipated increase in plasma T4 and T3 concentrations during cold exposure was blunted in iron deficiency. Post-exposure plasma norepinephrine concentration was increased significantly in iron deficiency.

Nutritional indices of iron status also responded, al-

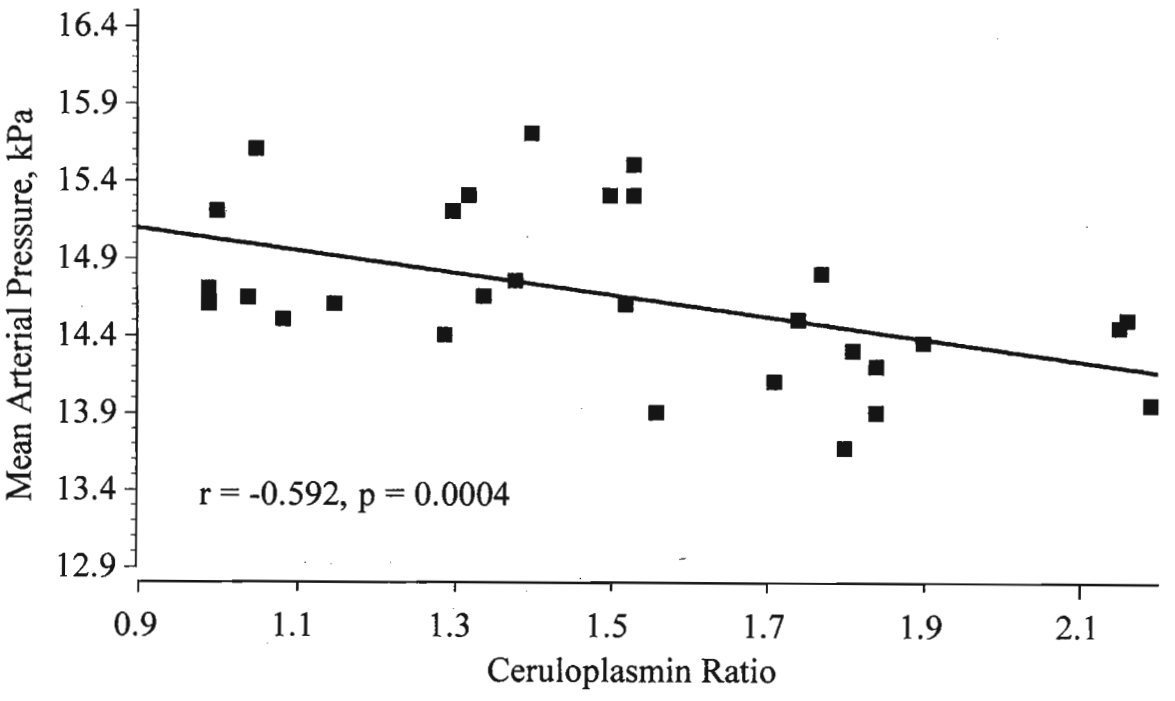

FIGURE 3 Relationship between individual mean arterial pressures after 5 min of sustained hand-grip work and the ratio of enzymatic to immunoreactive ceruloplasmin. Each symbol represents one volunteer. Used with permission from Lukaski et al. 1988.

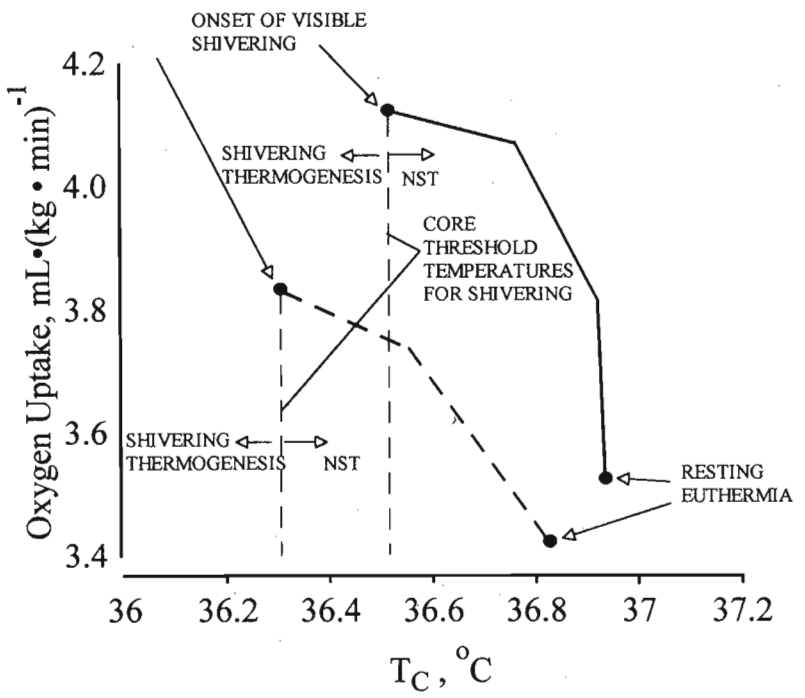

FIGURE 4 Relationship between oxygen uptake and mean body temperature (TC) of women experiencing acute cold exposure during iron deficiency and iron supplementation. Solid line represents iron-adequate and abbreviated line indicates iron-depleted responses. NST = nonshivering thermogenesis. Used with permission from Lukaski et al. 1990.

beit moderately, to the experimental interventions. Iron balance (−9.1 ± 2.6 vs. 28.9 ± 5.1 mg/d), hematocrit (0.355 ± 0.004 vs. 0.364 ± 0.004), hemoglobin (120 ± 20 vs. 126 ± 10 g/L) and serum ferritin concentration (5.5 ± 0.5 vs. 9.5 ± 0.4 ng/mL) increased significantly with iron repletion. However, these changes were modest compared with the marked changes in thermoregulatory parameters.

A comparison of thermoregulation in women with iron-deficiency anemia, depleted iron stores and iron-adequate status during immersion in 28°C water also revealed physiologic impairments associated with reduced iron status (Beard et al. 1990). The anemic, compared with the iron-adequate women had significantly lower rectal temperatures (36 ± 0.2 vs. 36.2 ± 0.1°C) and a decreased rate of oxygen uptake [5.28 ± 0.26 vs. 5.99 ± 0.29 mL/(kg·min)] after 100 min of immersion in the cold water. Plasma T4 and T3 concentrations were less in the anemic as compared to the iron-adequate women before and after immersion in the cold water. The physiological responses of the iron-depleted women were similar to those of the iron-adequate women. Iron supplementation of anemic women significantly improved body temperature response and partially normalized the thyroid hormone concentrations during the cold water exposure.

Further evidence of altered energy utilization in response to iron depletion can be found with the use of exercise as a stressor. In response to a graded, maximal exercise protocol on an ergocycle, iron-deficient but not anemic women failed to demonstrate reductions in peak oxygen uptake, cardiovascular or ventilatory function which are characteristic signs of anemia (Lukaski et al. 1991). Iron depletion, however, was associated with a significantly reduced rate of oxygen utilization, total oxygen uptake and aerobic energy production, and significantly increased peak respiratory exchange ratio and postexercise plasma lactate concentration (**Table 2**). These observations indicate that reductions of body iron without anemia affect energy metabolism by reducing total aerobic energy production and increasing glycolytic metabolism.

Ethanol metabolism and zinc

Another experimental strategy is to determine in vivo the effect of altered dietary intake of a mineral element on the rate of clearance or utilization of an administered substrate which is metabolized by a specific metal-containing enzyme. This approach provides an indirect assessment of the activity of the mineral-containing enzyme(s) in response to controlled intake of the mineral necessary for enzyme activity. A useful example is the influence of dietary zinc on ethanol metabolism in humans.

The activity of liver alcohol dehydrogenase (EC 1.1.1.1), a zinc-containing enzyme, was assessed indirectly during an ethanol tolerance test in a group of five postmenopausal women fed varied amounts of dietary zinc (Milne et al. 1987). A standardized dose of ethanol (0.5 g/kg body weight) mixed into orange juice was ingested after consuming control (6.73 mg zinc/d for 24 d), zinc-depletion (2.64 mg zinc/d for 125 d), and zinc-repletion (31.4 mg zinc/d for 27 d) diets. The time course of venous ethanol concentrations indicates that restricted dietary zinc may impair the metabolism of ethanol (**Fig. 5**).

Indices of alcohol metabolism measured during the ethanol tolerance test were affected by dietary zinc (**Table 3**). Approximately twice as much ethanol ap-

TABLE 2

Effects of iron status on peak oxygen uptake (VO_2), peak carbon dioxide output (VCO_2), respiratory exchange ratio (RER), total oxygen uptake (VO_{2tot}), total energy expenditure (TEE), and plasma lactate concentration ([lac]) during graded, maximal ergometer work[1]

	Control	Depletion	Repletion
Time, *min*	18.4 ± 0.6	18.3 ± 0.5	18.7 ± 0.6
VO_2, *L/min*	1.84 ± 0.07	1.78 ± 0.08	1.87 ± 0.08
VCO_2, *L/min*	1.91 ± 0.06	2.11 ± 0.09†	2.02 ± 0.08
RER	1.06 ± 0.01	1.13 ± 0.02†	1.06 ± 0.01
VO_{2tot}, *L*	17.1 ± 1.1	15.8 ± 1.2†	17.4 ± 1.0
TEE, *kJ*	467 ± 12	422 ± 10†	476 ± 10
[lac], *mmol/L*	12.6 ± 1.1	16.2 ± 1.3†	12.7 ± 1.0

[1] adapted from Lukaski et al. 1991.
† Significantly different ($P < 0.05$) by repeated measures ANOVA and Tukey post-hoc test.

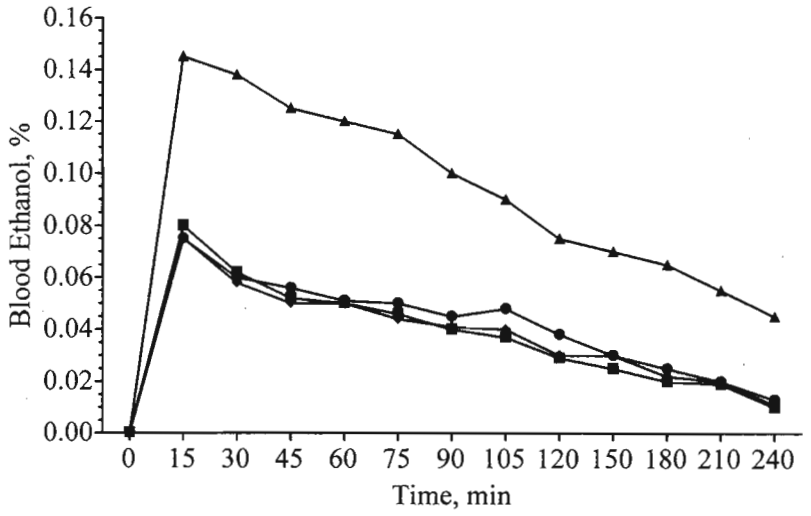

FIGURE 5 Blood ethanol concentrations in women fed different amounts of dietary zinc (Zn). Square = 6.73 mg Zn/d, circle and triangle = 2.64 mg Zn/d and diamond = 31.4 mg Zn/d. Used with permission from Milne et al. 1987.

peared in the blood within 15 min of ingestion at the end of the low zinc period compared with the end of control, the middle of zinc depletion and the end of repletion. Significantly more ethanol remained in the blood 4 h after ingestion when zinc intake was restricted compared with an adequate or supplemental intake. Approximately 75% of the ethanol observed at the maximum ethanol concentration in blood was eliminated from the blood by 4 h at the end of the low zinc period. This compared with about 85% elimination for the other dietary periods. The area under the tolerance curve also was significantly greatest at the end of the zinc depletion period. The absolute ethanol clearance rate was significantly greater at the end of the low zinc period. The rate of ethanol clearance, however, apparently was concentration dependent (Fig. 5, Table 3). When a concentration-independent rate constant for ethanol clearance was determined (Table 3), there was a reduction (28.9%) in the rate of ethanol clearance during the low zinc period and an increase (36.5%) during zinc repletion. Thus, zinc depletion was associated with impaired ethanol clearance.

Standard biochemical measures of zinc status were generally unresponsive to the manipulations in dietary zinc. Plasma zinc concentration did not change from control to zinc restriction (11.9 ± 0.4 vs. 12.9 ± 0.8 μmol/L) but increased slightly with zinc supplementation (13.2 ± 0.5 μmol/L). There were no changes in the zinc content of blood cellular components or zinc-containing enzymes, including alkaline phosphatase (EC 3.1.3.1), total carbonic anhydrase (EC 4.2.1.1), δ-amino-levulinic acid dehydratase (EC 4.2.1.24), and angiotensin converting enzyme (EC 3.4.15.1) in response to dietary zinc. Zinc balance data were variable. There was a nonsignificant decrease from 0.17 ± 0.12 to 0.07 ± 0.03 mg/d from control through depletion, but a significant increase to 0.90 ± 0.33 mg/d with zinc supplementation. Thus, while homeostatic mechanisms maintain circulating zinc concentrations, zinc and the activity of zinc-containing enzymes in tissues may be decreased before changes in circulating zinc concentrations are observed. Functional indicators of zinc biochemistry, such as ethanol metabolism, may be more sensitive to changes in zinc stores and nutritional status than circulating zinc concentrations.

Similar alterations in ethanol metabolism were observed in young men fed dietary zinc in amounts of 1, 2, 3, 4 and 10 mg/d for 36-d periods in a double-blind Latin-square design (Milne and Johnson 1993). Ethanol clearance was significantly decreased when dietary zinc was less than 10 mg/d. Standard biochemical measures of zinc status were not sensitive to the short-term changes in dietary zinc. These findings indicate that functional (e.g., ethanol metabolism) changes are more responsive to graded dietary zinc than static blood biochemical measurements of human zinc nutritional status.

TABLE 3

Blood ethanol (EtOH) variables during EtOH tolerance test in women fed graded dietary zinc[1,2]

Dietary zinc, *mg/d*	6.73	2.64	2.64	31.4
Day of study	22	51	141	171
Peak [EtOH], %	0.90 ± 0.03[a]	0.08 ± 0.02[a]	0.15 ± 0.02[b]	0.08 ± 0.01[a]
mmol/L	19.5	17.4	32.6	17.4
[EtOH] after 240 min, %	0.009 ± 0.008[a]	0.009 ± 0.004[a]	0.037 ± 0.021[b]	0.011 ± 0.007[a]
mmol/L	1.95	1.95	8.00	2.40
Area of tolerance curve, *min · %*	7.79 ± 2.17[a]	8.04 ± 1.31[a]	18.2 ± 2.60[b]	7.34 ± 3.08[a]
Apparent EtOH clearance rate (120–240 min), *mmol/(L · h)*	300 ± 73[a]	278 ± 63[a]	454 ± 80[b]	271 ± 67[a]
EtOH clearance rate constant,[3] *[mol/(mLpdh)/* [mmol/mL]]	38.9 ± 10.3	35.7 ± 4.4	28.9 ± 12.1	36.5 ± 6.6

[1] Adapted from Milne et al. (1987).
[2] EtOH values are means ± SD. [ab] Values in same row with different superscripts are significantly ($P < 0.05$) different.
[3] Concentration-independent rate constant calculated as the EtOH clearance rate between 120 and 240 min divided by the 120 min [EtOH].

PSYCHOLOGICAL FUNCTION AND PERFORMANCE

Broadly defined, behavior includes all aspects of psychological function and performance. In essence, behavior encompasses how we feel, think and act, how we perform routine as well as demanding tasks in our everyday lives, and how we relate to our physical and social environments. Behavior is unique as a criterion for evaluating nutritional adequacy because it involves the functional integration of all biological systems. Behavior is multifactorial and highly complex, supported by many physiological and psychological resource pools with sophisticated control (feedback) and compensatory mechanisms, which very often determine the practical importance of a nutritional deficit or excess for biological function.

Behavior includes mental and emotional processes as well as overt action. The mental aspect of behavior is most frequently referred to as cognition. Cognition is a collection of theoretically distinct but interdependent mental processes including sensation, attention, perception, learning, memory, concept formation, problem-solving and decision-making. It also includes skilled behaviors and response execution. Performance depends on the coordinated function of most or all of these processes. For experimental purposes, however, cognitive function may be assessed by measuring performance on highly structured tasks which emphasize a single or a few processes while minimizing the demands on all other processes to achieve successful performance.

Emotion is the affective aspect of behavior which includes mood states such as anxiety, depression and hostility, and other highly subjective states such as pain and sleep quality. Related behavioral phenomena are motivation and response style. These subjective states can be assessed by drawing inferences from overt behavior or by obtaining structured self-reports or reports from trained observers.

Because behavior results from a complex interplay of physiology, covert processes and subjective states, assessment of psychological function and performance can benefit from the complementary assessment of relevant physiologic function. The electrical activity of defined regions of the brain is sensitive to (e.g., reflects) ongoing mental processes and emotional states, and can be studied noninvasively and quantified by using electroencephalography (EEG). This methodology has proven useful in the evaluation of several general nutritional deficiencies and is potentially useful for assessing the effects of marginal and mild mineral element deficiencies on brain function. Knowledge of the effects of nutrition on brain electrical activity, and thus brain function, will provide valuable insights into the possible mechanisms underlying relationships between nutrition and behavior. Other issues relating nutrient intake or nutritional status to behavior and brain function are described elsewhere (Penland 1994).

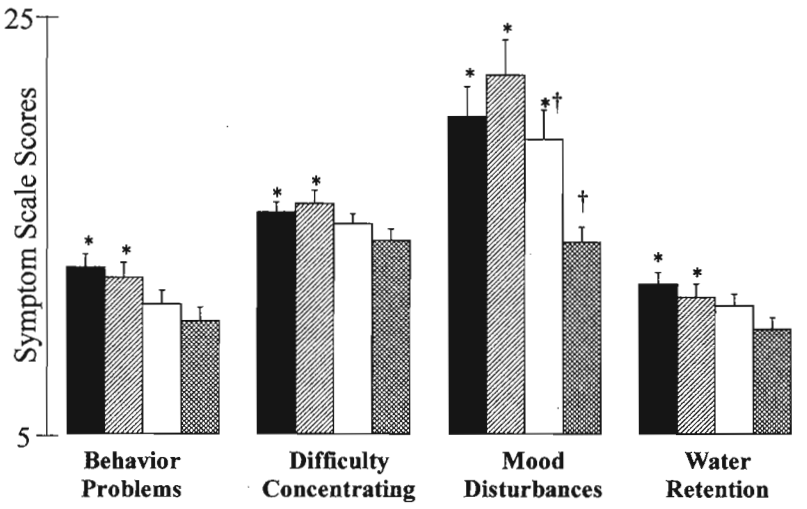

FIGURE 6 Menstrual Distress Questionnaire scores in relation to dietary calcium (Ca) and manganese (Mn) intakes: premenstrual phase. Fully shaded bars = 587 mg Ca and 1.0 mg Mn, partially cross-hatched bars = 587 mg Ca and 5.6 mg Mn, open bars = 1336 mg Ca and 1.0 mg Mn, and fully cross-hatched bars = 1336 mg Ca and 5.6 mg Mn. *Significant differences ($P < 0.05$) between treatments were identified by repeated measures ANOVA. †Significant interactions ($P < 0.05$) were identified with Bonferroni contrasts ($P < 0.05$). Adapted from Penland and Johnson 1993.

Menstrual cycle symptoms, calcium and manganese

The effects of supplemental calcium and manganese on self-reported symptoms during the menstrual cycle were examined in 14 women living on a metabolic unit (Penland and Johnson 1993). The women were assigned in a double-blind, Latin-square design to each of four 39-d periods created by the factorial combination of 587 and 1336 mg calcium/d and 1.0 and 5.6 mg manganese/d. An adaptation of the Menstrual Distress Questionnaire (Moos 1969) was administered individually to each woman at the completion of the menstrual phase of each cycle; the questionnaire provides a retrospective self-report of the presence and severity of symptoms occurring during the menstrual cycle.

In contrast to when they were fed the high calcium diet, women fed low calcium reported significantly increased mood disturbances (e.g., anxiety, loneliness, crying, depression, mood swings, tension) and more behavioral problems during the premenstrual and menstrual phases of the menstrual cycle (**Fig. 6, 7**). Low calcium intake resulted in reports of increased difficulty concentrating during the premenstrual phase and marginally ($P < 0.052$) during the menstrual phase. Low calcium also resulted in reports of greater pain during the menstrual phase and increased water retention during the premenstrual phase. An interaction between calcium and manganese was observed for mood disturbances and marginally ($P < 0.068$) for pain during

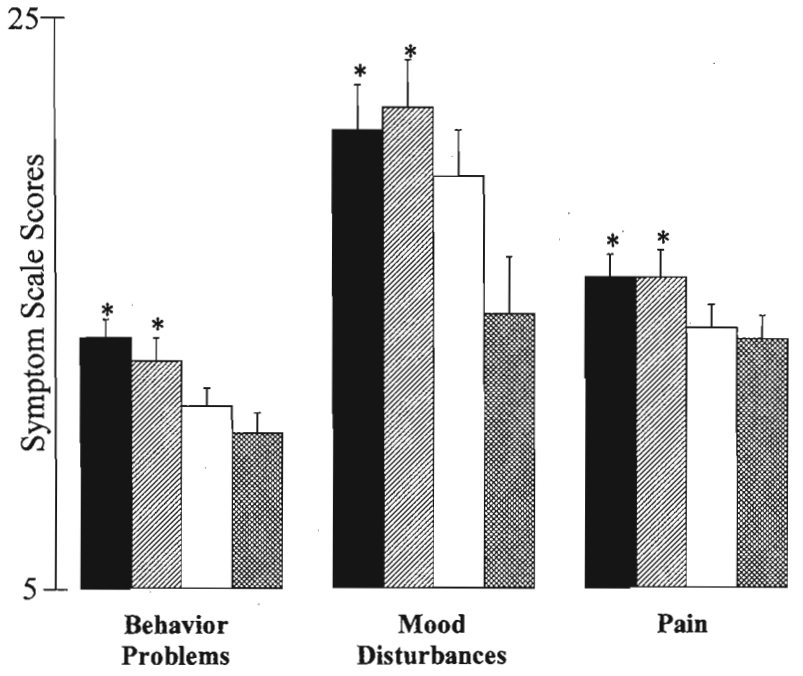

FIGURE 7 Menstrual Distress Questionnaire scores in relation to dietary calcium (Ca) and manganese (Mn) intakes: menstrual phase. Fully shaded bars = 587 mg Ca and 1.0 mg Mn, partially cross-hatched bars = 587 mg Ca and 5.6 mg Mn, open bars = 1336 mg Ca and 1.0 mg Mn, and fully cross-hatched bars = 1336 mg Ca and 5.6 mg Mn. *Significant differences ($P < 0.05$) by repeated measures ANOVA. Adapted from Penland and Johnson 1993.

the premenstrual phase. Similar effects of calcium intake on menstrual symptoms were observed in women suffering premenstrual symptom (Thys-Jacobs et al. 1989).

Nutritional status wasaffected to a small degree by the experimental diets (Johnson and Lykken 1991). Although calcium balance was positive at both dietary calcium intakes, it was significantly greater at the higher dietary intake. Menstrual losses of manganese, calcium, iron and hemoglobin were significantly greater when the women consumed the diets low in manganese.

Sleep patterns and dietary copper and iron

Sleep is a complex physiological and psychological phenomenon that apparently is influenced by dietary mineral element intake. Sleep quantity and quality were reported by women who resided on a metabolic unit, consumed diets varying in copper and iron contents, and completed the Sleep Behavior Inventory immediately upon awakening each morning (Penland 1988). Compared with when they consumed a diet adequate in copper (>2 mg/d), 11 women fed a low copper diet (< 1 mg/d) reported significantly earlier bedtimes, significantly longer latency to sleep, significantly longer total sleep time, and feeling significantly less rested upon awakening (**Table 4**). In contrast to when they were fed a diet containing adequate dietary iron (>15 mg/d), 13 women fed a diet low in iron (<5 mg/d) reported significantly earlier bedtimes, significantly more night-time awakenings and significantly longer sleep times. The increased total sleep time, observed during periods of low intakes of both copper and iron, may represent a major compensatory response. For example, sleep quantity may increase during times of illness and stress although sleep quality may decrease. These findings indicate that mineral element intake influences sleep behavior in adult women.

Cognitive performance and zinc

A topic that draws considerable public health interest is the effect of nutritional status on human cognition. The effects of severe and marginal iron deficiencies on the cognitive, behavioral and social functions of children have received considerable study. A detailed discussion of findings and pertinent research issues was presented at the International Conference on Iron Deficiency and Behavioral Development (Haas and Fairchild 1989).

The effects of graded zinc intake on cognitive function of men recently were examined. Healthy men responded to a battery of tasks assessing cognitive processes and psychomotor skills while living on a metabolic unit and consuming diets containing 1, 2, 3 and 4 mg zinc/d in a random, double-blind manner for 35-d periods, concluding with another 35-d period that provided 10 mg zinc/d (Penland, unpublished data). Although the findings indicate that moderate zinc deprivation of healthy men significantly affects a broad range of cognitive and psychomotor functions, tasks emphasizing attention, perception and memory were most affected. For an individual, relationships between graded zinc intakes and performance on specific tasks used in this study were not always unambiguous. That is, no single task was uniformly affected by dietary zinc. This finding underscores the fact that behavior and cognitive processes are highly complex and interdependent. When confronted with a nutritional stressor such as low dietary zinc, it is very likely that overall

TABLE 4

Effects of dietary copper and iron on self-reported sleep patterns of women[1]

	Copper		Iron	
	mg/d			
	<1.0	>2.0	<5	>15
Evening bedtime	352†	364	320†	349
Latency to sleep onset	18†	16	11†	9
Night-time awakenings	0.60†	0.51	0.71†	0.59
Total sleep time	358†	346	396†	370
Sleep quality	1.91†	1.97	1.99	1.96

[1] Adapted from Penland (1988).
† Significantly different ($P < 0.05$) than corresponding value for same mineral.

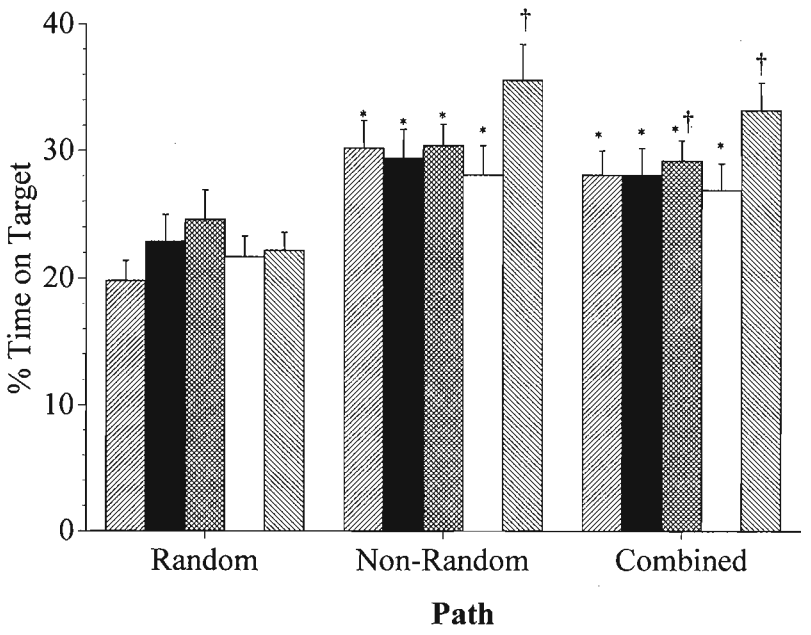

FIGURE 8 Effects of dietary zinc (Zn) on psychomotor function: pursuit task. Left cross-hatch = 1 mg Zn, solid bars = 2 mg Zn, full cross-hatch = 3 mg Zn, open bars = 4 mg Zn and right cross-hatch = 10 mg Zn. *†Bars with different symbols are significantly different ($P < 0.05$) by repeated measures ANOVA. Adapted from Penland (unpublished data).

performance may not be affected because compensatory mechanisms that utilize unused resources are invoked; the resource pool of zinc is not exhausted. This compensation, however, is incomplete across all task components. This point is emphasized by the finding that the supplemental zinc period (10 mg zinc/d), compared with the low dietary zinc periods, was associated with improved psychomotor performance as indicated by the increased success in tracking an object following a nonrandom path on a computer screen (**Fig. 8**). Thus, the use of multiple task conditions to assess nutritional effects on psychological performance enhances the ability to detect functional consequences.

Brain electrical activity and magnesium

The effects of dietary magnesium (115 and 315 mg/d) on electroencephalographic (EEG) activity were examined in 13 postmenopausal women residing on a metabolic unit (Penland 1995). Compared with high dietary magnesium, the low magnesium intake significantly increased total power in the frontal regions and the right temporal and parietal regions, and resulted in frequency-specific increases in the left occipital delta power, theta power in all but the left temporal region, alpha power in the right frontal and right temporal regions, and beta power in the frontal regions (**Fig. 9**). The proportion of theta power to total power in the parietal regions also increased with the low magnesium diet. These effects of low dietary magnesium were observed principally when the women had their eyes closed. The findings of increased central nervous system activity during short-term magnesium deprivation in these older women are consistent with the hyperexcitability seen following experimentally induced magnesium deprivation and magnesium deficiency in humans. Thus the use of quantitative analysis of brain electrical activities can provide unique information regarding brain function in response to altered nutrient intake.

IMMUNE FUNCTION

The concept that mineral nutritional status may play a role in regulating the susceptibility of a host to infections is not new (Kramer and Johnson 1993). Although general information describing connections between environmentally induced perturbations in mineral nutritional status and immune status of livestock is available, there is a void of information relating immune responses during controlled metabolic studies of healthy humans (Prohaska and Failla 1993). Such studies are required to clearly define relationships between mineral intake and host responses independently of complications associated with genetic abnormalities and pathology.

Human immune response and copper

Indices of immune response were examined in 11 men fed diets containing 0.66, 0.38 and 2.49 mg copper/d for 24, 42 and 24 d, respectively (Kelley et al. 1995). The low copper diet (0.38 mg/d) was associated with a significant decrease in proliferation of peripheral blood mononuclear cells cultured in vitro with specific mitogens, including phytohemagglutinin, concanavalin A and pokeweed. This diet also elicited a significant increase in the percentage of circulating B cells. Other indices of immune response hypothesized to respond to copper deprivation (e.g., serum interleukin 2 receptor and neutrophil phagocytic activity) were unaffected by dietary copper. Consumption of increased dietary copper (2.49 mg/d) prevented further decreases in the immune response indicators influenced by the low copper diet but did not restore them to control values. Blood biochemical indices of copper status (plasma copper and ceruloplasmin concentrations), however, did respond to the increase in dietary copper from 0.38 to 2.49 mg/d.

An interesting example of the relationship between copper deficiency and immune status in adult humans was recently reported (Smith et al. 1994). Two adult patients, one male and one female, with anemia and neutropenia were found to have abnormally reduced concentrations of copper and ceruloplasmin protein in plasma, and decreased erythrocyte superoxide dismutase (EC 1.15.1.1) and platelet cytochrome c oxidase (EC 1.9.3.1) activities in conjunction with impaired in vitro lymphocyte blastogenesis in response to conca-

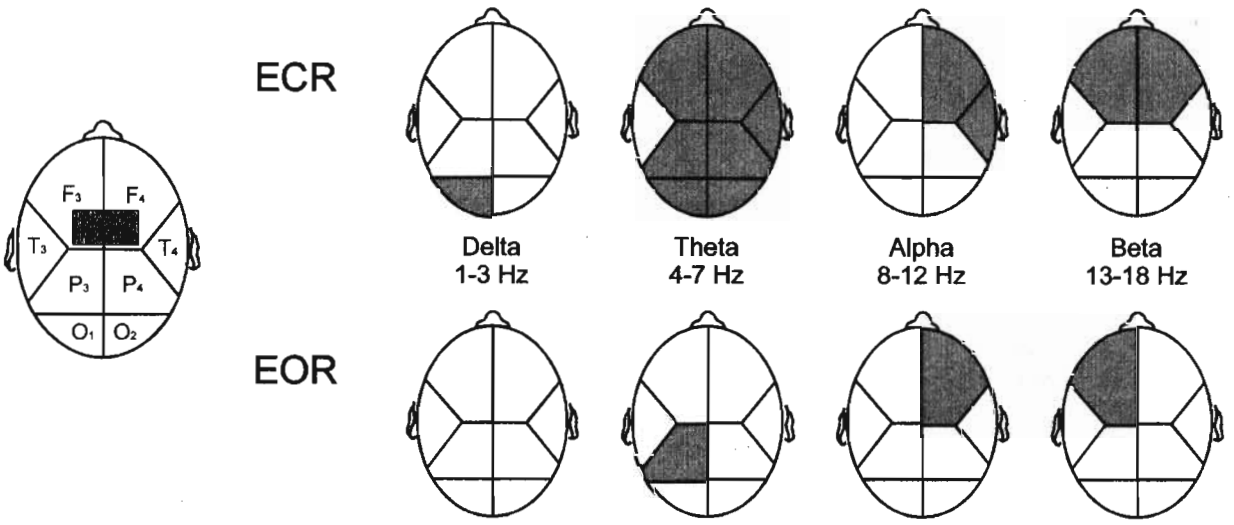

FIGURE 9 Summary of the effects of dietary magnesium (115 vs. 315 mg/d) on electroencephalographic spectral power during eyes-closed (ECR) and eyes-open (EOR) at rest. Shaded areas indicated significantly ($P < 0.05$) greater power with 4.8 mmol magnesium intake. F, T, P and O refer to electrode placements in frontal, temporal, parietal, and occipital areas, respectively, of the skull. Adapted from Penland 1995.

navalin A and phytohemagglutinin. Intravenous treatment with copper restored immune response and blood biochemical measurements of copper nutriture in one patient. The second patient, however, was treated with oral copper, as copper gluconate, which after 4 mo only partially improved the blood biochemical indicators of copper status and the mitogenic response. This observation suggests that some indicators of immune function are responsive to impaired copper nutritional status in humans and respond positively to copper supplementation.

SUMMARY AND CONCLUSIONS

The challenge of identifying experimental strategies that are useful in refining the estimates of dietary intake of the mineral elements is considerable. One salient point is that previous approaches, which included reliance on chemical balance methods and measurement of circulating mineral concentrations, have been successful in some cases in identifying individuals with severe mineral deficiencies but are not capable of discerning mild deficiency states that are perhaps more prevalent than presently identified.

The imminent need for novel approaches is apparent. If one accepts that nutritional requirements should be based on the objective of optimizing health, then one may propose that assessments of biological function may be a potentially useful experimental tactic. Theoretically, the requirement for any mineral element should be defined by the ability of a cell, tissue, organ, system, and organism to function optimally. If one can identify a specific biological function, or an integrated system of functions, that is dependent on a single mineral element, then one can examine the function as a diagnostic test. This approach has been used to demonstrate altered physiological and psychological (behavioral and cognitive) responses to restricted intakes of calcium, iron, copper, magnesium and zinc in controlled human studies. In addition, this paradigm may be very effective in assessing the effect of restricted mineral intakes on the expression of gene products such as proteins and enzymes.

The application of functional assessment of human nutrient requirements is, at this time, restricted to experimental situations in which this information is part of a broad series of measurements, biochemical and functional. In this manner, functional assessments may continue to be evaluated for specificity and sensitivity in responding to controlled alterations in mineral intake.

It is anticipated that future research will attempt to further elucidate the value of biological function assessments in conjunction with traditional and new biochemical and molecular biology assessments of mineral nutriture to determine human mineral element requirements. Such efforts should allow more complete and definitive estimates of human mineral requirements based on individual needs.

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